Docket No.: NBI-193
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Xianqi Kong *et al.*

Application No.: 10/763,953 Confirmation No.: 5062

Filed: January 23, 2004 Art Unit: 1614

For: AMIDINE DERIVATIVES FOR TREATING AMYLOIDOSIS Examiner: J. M. Nolan

DECLARATION UNDER 37 C.F.R. §1.131 BY XIANQI KONG, XINFU WU AND DAVID MIGNEAULT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

We, Xianqi Kong, Xinfu Wu, and David Migneault declare:

1. We are the inventors of the subject matter described and claimed in the above referenced patent application.

2. Prior to March 6, 2003, the invention described and claimed in the above-referenced application was completed in Canada, as evidenced by the following:

Copies of notebook pages evidencing the synthesis of the claimed compounds, attached hereto as Appendix A.

The undersigned hereby declare that all statements made herein of their own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: Oct. 25, 2006 Signed: _____
Xianqi Kong

Date: Oct. 25, 2006 Signed: _____
Xinfu Wu

Date: Oct 26, 2006 Signed: _____
David Migneault

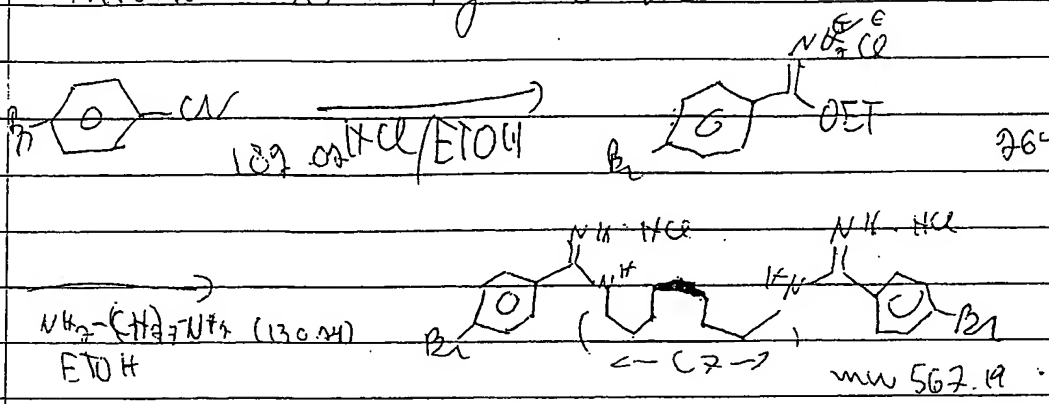
APPENDIX A

Neurochem Inc.

DM-191-114

18/07/02

Internal bis benzamidine



4-Bromo benzonitrile (0,91 g, 5 mmol) was loaded in a 100 mL RBF - flushed with ~~ethanol~~ Nitrogen, + ethanol (≈ 25 mL) Cooled to 0°C saturated with dry HCl (g). Stirred at r.t (ON. 13 h). DM

19/07/02

Work Rx: IR: no S.M. left. - Solvent dried in vacuo after ether precipitation. 1,21 g (4.57 mmol, 91%) Very clean ^{1}H NMR.

→ A solution of 1,7-diamino heptane (257 mg, 1,97 mmol) in ETOH (10 mL) was added to a dispersion of the amidate in ethanol (5 mL). Stirred at r.t. Solid rapidly began to form in the clear initial solution. DM

22/07/02

Solid: 10 mg of solid in suspension. Filtered out, dried. ML was heated to reflux 1,5 h, - solvent. Some solid

r.t 8 h

Go to 18C

Signature	Read and Understood (print)
	ISABELLE VALADE
	Isabelle Valade
Date (D/M/Y)	Signature 01/08/2002 Date (D/M/Y)

Neurochem Inc.

AM-91-14 (from p 177) 22/07/02

→ + ether: gummy solid/wax at the bottom: - solvent.

Solid from r.t: 66.8 mg. ✓ discarded. 24/07/02

Dissolved in conc HCl (≈3 ml, hot) + acetone. Sln placed at -20°C. No solid: + ether: ≠ phase: diluted with water, decanted ether layers was discarded. Aq HCl layer extracted 3x CHCl3 (75 ml). Concentrated in vacuo. Aq phase was also concentrated in vacuo. PM 25/07/02

CHCl3 extract: 0.16 g
HCl/water layer: 0.76 g

CHCl3 check by MS or HPLC: try
HCl: try to clean by solvent: Cryst/free. PM 29/07/02

24/7/03 A very small amount of solid was collected and dried in vacuo. The Filtrate was concentrated and dried in vacuo: foamy solid 720 mg: tested by LC: many peaks: wait for Prep LC Time.

Signature		Read and Understood (print)
		ISABELLE VALADE
Date (D/M/Y) 31/07/02		Signature 01/08/2002 Date (D/M/Y)

Gu 10 180

Neurochem Inc.

NM-191-114 (From p180) 01/08/02

b: 14.2060 → Test LC run : Late Fractions (~42 min : MS : Product: (NM-191-114-b).

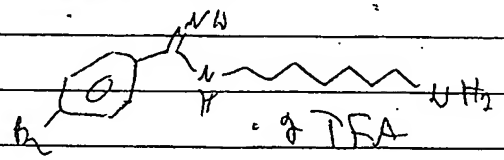
a: 23.2138 → An earlier peak showed mono-derivatized amine (NM-191-114-a).

a: concentrated: not very soluble in water.

a: 35 mg

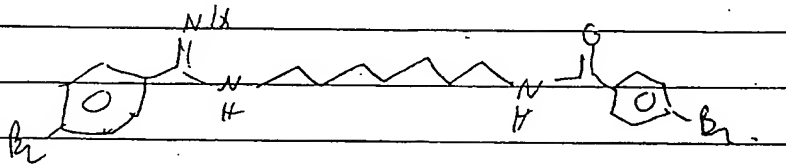
b: 23.7mg (desired Material)

a:



: 2 TFA

b) ^1H NMR: suggest



Lane a: 13.4438 b: 13.4884 → a and b Transferred into amber vial and were freeze-dried DM 06/08/02

a: 29.2 mg b: 18.7 mg.

Sun milled

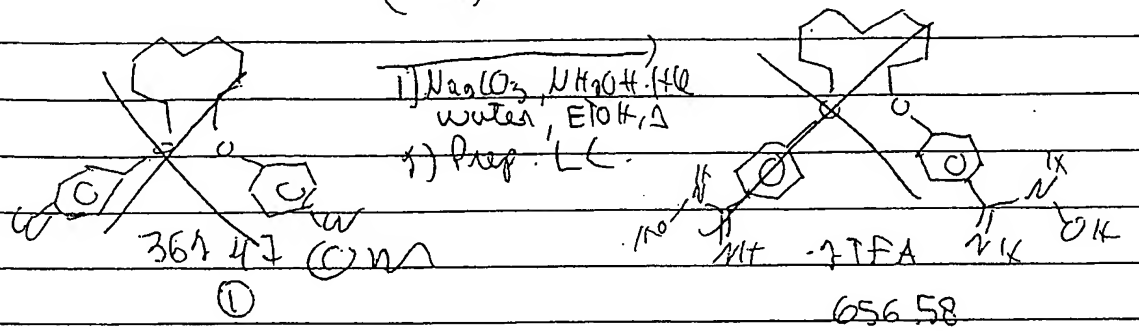
Signature	Date (D/M/Y)
O Mignault	07/08/02

Read and Understood (print)	Signature	Date (D/M/Y)
ISABELLE VALADE	Isabelle Valade	11/09/2002

Neurochem Inc.

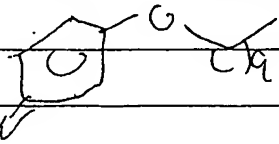
DM-191-136 21/08/02

Hydroxy imino (Cq)



428.53 A Mixture of the bis benzonitrile ① (181 mg, 0.5 mmol), Na_2CO_3 (180 mg) and hydroxylamine hydrochloride (280 mg) in 70% H_2O/EtOH (10 mL) was heated to reflux for 2h. 36.13%. Cooled to r.t. some solid was removed by filtration. The residue was dried in vacuo. 400 mg. 79% by 1H NMR DM 23/08/02.

Prep LC: main peak not desired product. Verified lot WL-159-135: was not bis benzonitrile:



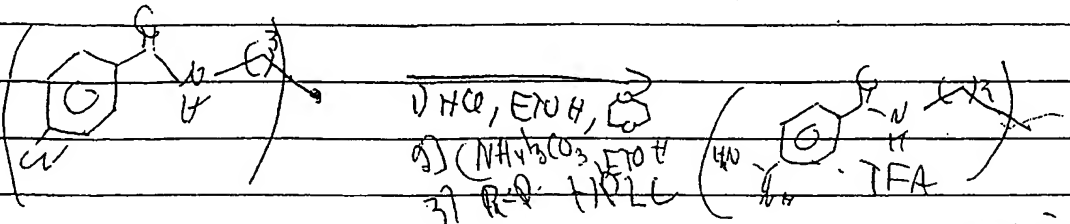
→ DM-191-136-Q dried in vacuo.

Time: 34.7713 Correct for FOM SM WL-159-031. DM 23/08/02

48.5mg Rob Submitted

Signature	Read and Understood (print)
Date (D/M/Y) 30/08/02	Signature 25/10/02 Date (D/M/Y)

C7 - bis ~~Boc~~ amidino benzamidate



major.

The crude benzonitrile (DM-191-139, 1 mmol) was suspended in 1,4-dioxane (15 mL) / EtOH (6 mL). Saturated with HCl at 0°C Stirred at r.t. For the weekend sealed with a septum.

DM
27/08/07

FTIR: no nitrile left. Solvent was evaporated. Solid dried in vacuo, dissolved absolute ethanol (25 mL) : +3g $(NH_4)_2CO_3$. Stirred at r.t.

DM
28/08/07

After 2 h: Filtrat and solvent was evaporated. 0.5 g.

DM.
29/08/07

Purified by RP-Prep HPLC. 2 products (majors) were collected, concentrated and Freeze-dried

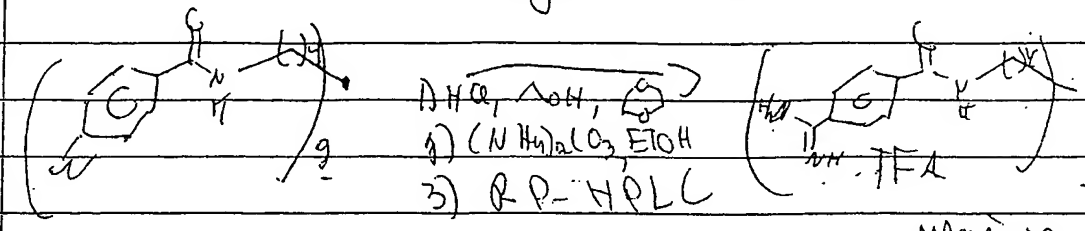
Signature	Read and Understood (print)
Date (D/M/Y) 29/08/07	Signature 13/09/07 Date (D/M/Y)

Neurochem Inc.

DM-191-143

23/08/02

(9- Bis amidino Benzamidate



Major-

The crude benzonitrile (DM-191-140 1 mmol) was suspended in 1,4-dioxane (17 mL) / EtOH (6 mL). Saturated with HCl at 0°C. Stirred at r.t for the weekend sealed with a septa.

DM

27/08/02

No nitrile left. Solvent was evaporated. Solid dried in vacuo. Dissolved in absolute ethanol (35 mL). 2g of ammonium carbonate was added (10. 13:15). Stirred at r.t.

29/08/02

After 24h; Filtered and solvent was evaporated. 0.54g. DM

29/08/02

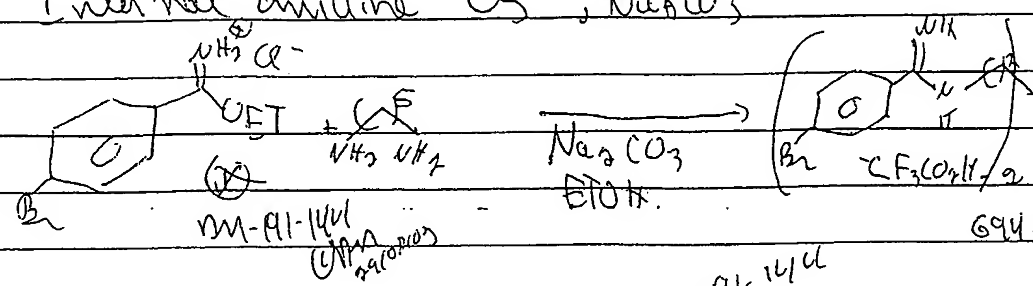
Purified by RP-Prep HPLC. 2 major products were collected, concentrated and Freeze-dried.

Signature	Read and Understood (print)
Date (D/M/Y) 29/08/02	Signature 13/09/02 Date (D/M/Y)

Neurochem Inc.

DM-191-145 29/08/02

Internal amidine C5, Na_2CO_3



Amixture of amidate (DM-191-144) (465 mg, 1.7 mmol, 1,5-diamino pentane (0.65 mmol, 77uL) and sodium carbonate (1.3g) in ethanol (10 mL) was stirred at room temperature. to - 13h15. DM.

30/08/02

filtered after 21h, solvent was evaporated.

2.3234 553mg. MS and HPLC one major component: Mass Fit. DM

03/09/02

1H NMR: major component also visible. DM.

04/09/02

Purified by RP-HPLC: collector did not worked. Main peak (MS: desired material) was collected in 2 fractions (Verified by analytical HPLC: pure).

goto 223 a 0.18g. b: 0.01g. Combined.

Lyophilized.

+ one

30 mL

48.0614

Signature	Read and Understood (print)
D. Migneault	Wenzhao Lu
Date (D/M/Y) 04/09/02	Signature [signature] Date (D/M/Y) 13/09/02

CONFIDENTIAL
ATTORNEYS' EYES ONLY

Neurochem Inc.

DM-191-142 (From pg 15) 30/08/02

a: 217.3 mg: desired product ^{1}H NMR

b: 79.9 mg: ester / amidine. ^{1}H NMR

a: mw 650.55 : 0.334 mmol, 33%.

b: mw 586.58 : 0.141 mmol 14%

MS ok for Both.

Submitted

Signature	
Date (D/M/Y) 30/08/02	

Read and Understood (print)	
Signature 13/09/02	Date (D/M/Y)

220

Neurochem Inc.

DM-191-143 (From 216) 30/08/02

a: 191.7 mg. desired product ^{1}H NMR

b: 96.5 mg. ester/amidine ^{1}H NMR

a: mw 678.63 : 0.282 mmol, 28 %

b: mw 594.63 : 0.162 mmol, 16 %

MS: OK for Both. Submitted.

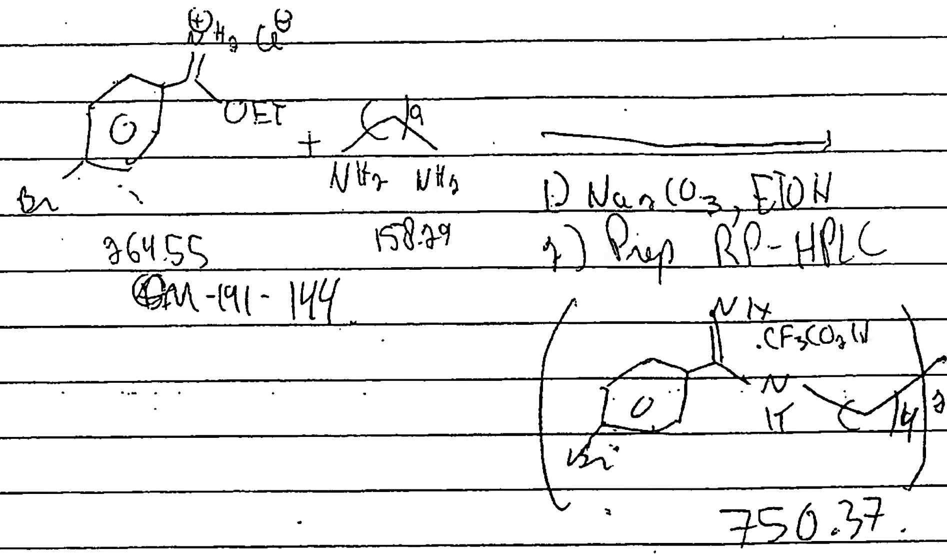
Signature	
Date (D/M/Y)	30/08/02

Read and Understood (print)	Wenshuo Lu
Signature	13/09/02 Date (D/M/Y)

Neurochem Inc.

DM-191-147 03/Aug/02

4-Bromo internal amidine, C9



A mixture of the amidate DM-191-144 (500 mg, 1.89 mmol), 1,9-diaminononane (108 mg, 0.68 mmol) and sodium carbonate (1.35 g) in ethanol (14 mL) was stirred for a day at room temperature. M.

04/09/02

22 h. Mixture was filtered, solid washed with methanol. Filtrate was concentrated to dryness. An aliquot was verified by RP HPLC (anal.). M.

05/09/02

0.67 g. Purified by RP-HPLC (prep). Freeze-dried M.

06/09/02

113.5838 231.3 mg, 0.308 mmol, 47% ¹H NMR, MS etc: Submitted.

113.8151

Signature	Read and Understood (print)
D. Migneault	Wenshuo Lu
Date (D/M/Y) 06/Sept/02	Signature [signature] 13/09/02 Date (D/M/Y)

Neurochem Inc.

DM-191-145 (From p218) 05/09/02

O. Mirgorodskaya

246.6 mg white solid: 0,355 mmol,
54% yield

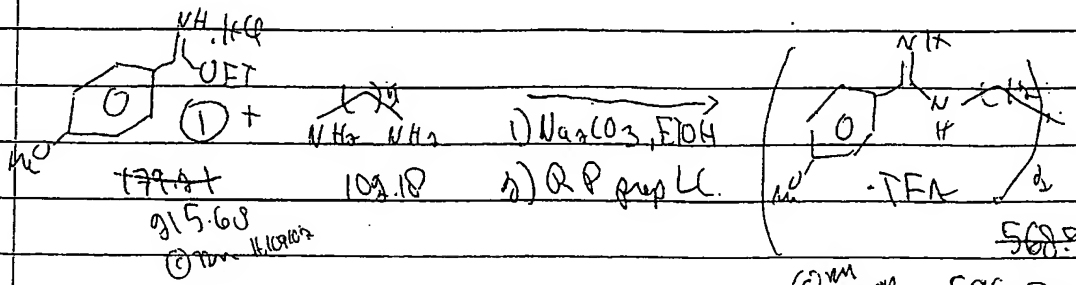
^{1}H NMR and MS ok: Submitted.

Signature	Read and Understood (print)
O. Mirgorodskaya	Understood by
Date (D/M/Y) 05/09/02	Signature 13/09/02 Date (D/M/Y)

Neurochem Inc.

PM-191-155 17/09/02

4-MeO, C5, internal amidate



A Mixture of 1,5-diamino pentane (100ul, 0.84 mmol), Sodium carbonate (1.45g) and the amidate 1 (400mg 2.23 mmol) in ethanol (10 mL) was stirred at r.t. (To: 11:05). DM

17/09/02

After 24: Filtered aliquot on MS and HPLC: ~~no~~ desired material present. Filtrate was concentrated to dryness and the residue was dried in vacuo. DM

16/09/02

Purified By RP-HPLC & Freeze-dried. DM

17/09/02

Vial weight 43.8812 - 43.5938

2874mg, ~~0.506 mmol~~ ~~60%~~

0.482 mmol, 57%

NMR - not pure. To repurify.

Signature	O. Miquel
Date (D/M/Y)	18/04/02

Read and Understood (print)	ISABELLE VALADE
Signature	Isabelle Valade
Date (D/M/Y)	18/10/2002

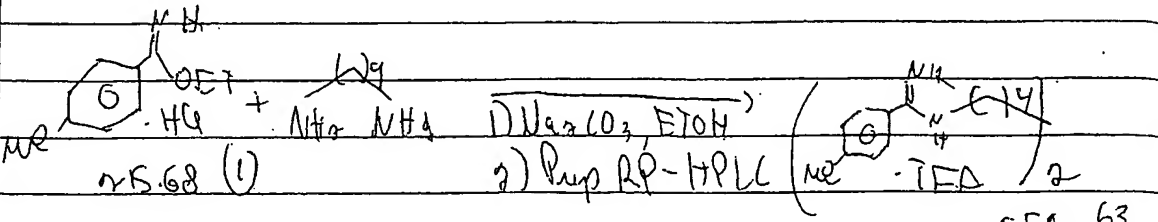
dc10
237

Neurochem Inc.

DM-191-157

16/09/02

4-MeO, C9, Internal amidine



n5.68 (1)

652.63

A mixture of the amidate (1) (0.55 g, 2.5 mmol), 1,9-diaminononane (159mg, 1mmol), Na_2CO_3 (1.5g) in ethanol (10 mL) was stirred at r.t for 24h. DM

17/09/02

Mixture was filtered. Solids were washed with MeOH (50mL). Filtrate was concentrated to dryness. Purified by R-P HPLC. Manual collection. Freeze-dried. DM

18/09/02

949.3 mg, 0.387 mmol, 38% Very Hygroscopic. 100% (HPLC). Very clean ^1H, ^{13}C NMR.

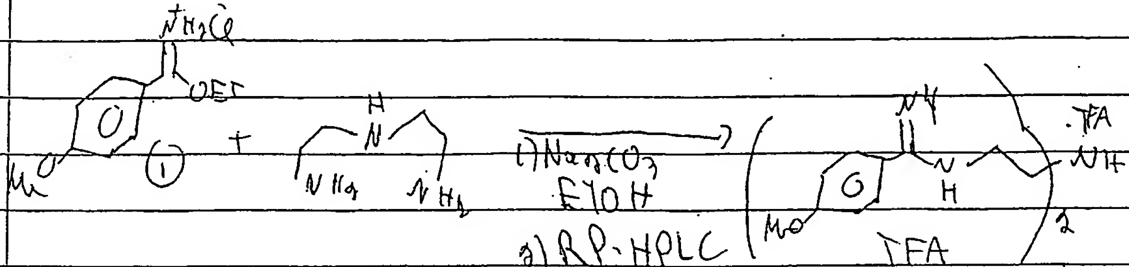
Submitted.

Signature	Read and Understood (print)
O. Mignonat	ISABELLE VALADE
Date (D/M/Y) 18/09/02	Signature Isabelle Valade 18/10/2002 Date (D/M/Y)

Neurochem Inc.

DM-191-159 17/09/02

Diethylenetriamine internal 4-MeO amidine.



711.54

A mixture of (1) (2.0 mm, 0.47g) diethylenetriamine (1 mmol, 90 μL) and sodium carbonate (145 g) in ethanol (10 ml) was stirred at r.t. for a day. DM

18/09/02

Filtered, + MeOH (50 ml). - solvent. DM

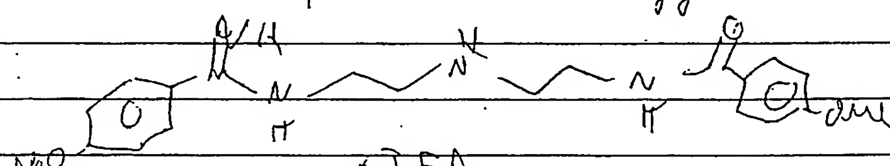
19/09/02

Purified by R-P HPLC, freeze-dried.

20/09/02

177.4 mg, 0.296 mmol.

^1H and ^{13}C, Cosy NMR suggest:



· 2 TFA.

MW = 598.50

Coevaporate 4× 95% MeOH. Dried in vacuo.

135818 MS: No: Spectrum in DMSO. 4 D exchange value H-

9070

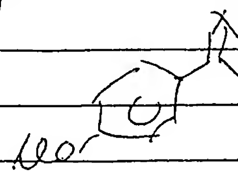
238

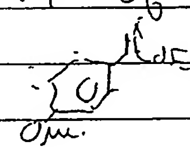
Signature	Read and Understood (print)
D. Migneault	ISABELLE VALADE
Date (D/M/Y) 20/09/02	Isabelle Valade Signature 18/10/2002 Date (D/M/Y)

DM - 191 - 155 (From p 237) 19/09/03

Re purified by prep R-P HPLC.
freeze dried DM
20/09/03

134.3 mg : ^1H NMR : still [illegible]

of  : X = N+ or O.

Recombined with left over fractions
- ACN (40 ml) aq. phase extracted
3x10 mL CH_2Cl_2, 1x 20 mL ether. Aq. layer
~~1.7718~~ was concentrated. 110 mg [illegible] of
0.7185 
.184 Attempt to recrystallize:
2N HCl, [illegible] EtOH
0.5 mL / 5 mL 0.5 mL DM
01/10/03

Crystals were collected by filtration, rinsed with
3.5417 acetone dried in vacuo. 37.6 mg pure
.5793 by ^1H, ^{13}C NMR.

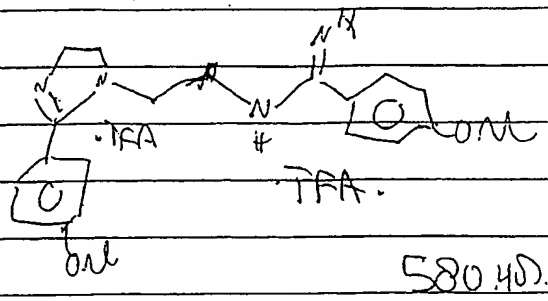
MW: 441.40 (0.085 mmol, 10% yield)
Committed

Signature	Read and Understood (print)
[signature]	ISABELLE VALADE
Date (D/M/Y) 28/10/03	Isabelle Valade Signature 10/12/2003 Date (D/M/Y)

Neurochem Inc.

M-191-159 (From p 236)

30/09/02



580.40.

156.6 mg

Total yield 177.4 mg
0.306 mmol

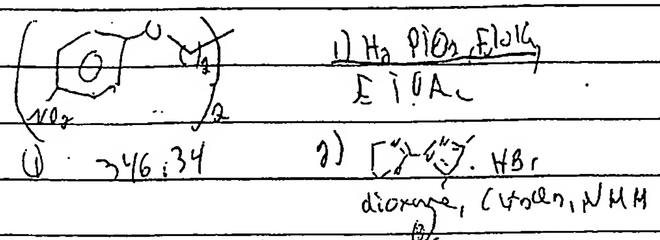
Clear glassy (soft) solid. 31%.

Submitted

Signature	Read and Understood (print) ISABELLE VALADE
Date (D/M/Y) 30/09/02	Signature 18/10/2002 Date (D/M/Y)

DM-191-168 07/10/02

C5, C5 linker, guanidine: 2eq NMM



The bis nitro (1) (176 mg, 0.508 mmol) was reduced with PtO2 (14mg) [H2 (55psi)] in a mixture of EtOH/EtOAc (1:1, 6mL), for 4h, at r.t. Rx completed (TLC). Filtered over Celite, -solvent. Residue dried 30 min in vacuo. Dissolved in a mixture of 1,4-dioxane (5mL), dichloromethane (1.5mL). Base: (NMM, 130 uL) Followed by (3) (270 mg, 1.02 mmol). Stirred at r.t, N2, protected from light. DM

NMM 1.14 mw 1.15 M, 107 ... (ml/mg)

08/10/02

Aliquoted for MS. No Rx. After 20h rt: oil bath at 60°C (-CH2Cl2). DM

09/10/02

Aliquot verified by MS (08h00). SM gone: mono derivatized. DM

10/10/02

Liq Yaro 575345

Δ 8h no change + charcoal, Filtered: Filtrate was concentrated to dryness. Tan: 79 N HCl: Filtered, -solvent, dried.

Tan: 59 4141

... 451

Signature: [signature]

Date (D/M/Y): 10/10/02

Read and Understood (print): ISABELLE VALADE

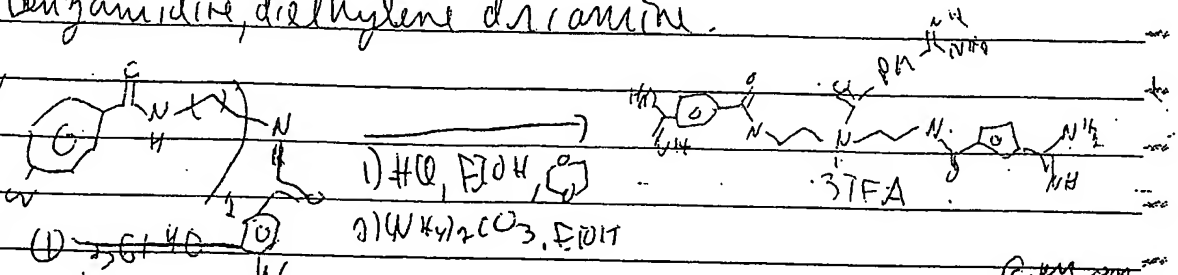
Signature: [signature] 18/10/2002 Date (D/M/Y)

Neurochem Inc.

DM-191-170

08/10/07

Benzamidine, diethylene triamine.



~~1) 3,6H4O~~
44.05%

Crude nitrile 1 (DM-191-166, ≈ 260 mg, ~~0.7~~ mmol) in ethanol (10mL), 1,4-dioxane (5mL), was saturated with dry HCl at 0°C. Stirred O/N at r.t (70 : 1 : 30).

09/10/07

No C≡N detected by FT-IR. concentrated to 4mL, + ether : solid collected rinced ether, dried in vacuo. Dissolved in EtOH (10mL). Ammonium carbonate (1.2g) was then added. Stirred at r.t.

13.9.07

Filtered, dried, purified by prep LC. Mass product m/z : 271. Dried in vacuo.

11/10/07

129 mg white solid. 0.146 mmol
94% - deuM MS. Trisubstituted
oic by 1H, 13C NMR : 95% pure -
Submitted.

Signature	D. Margetts
Date (D/M/Y)	4/10/07

Read and Understood (print)	ISABELLE VALADE
Signature	Isabelle Valade
Date (D/M/Y)	18/10/2007

NM-PI-168 (From p 247) 11/10/03

- ML: 111.4 mg (Liq)
- Tar: 760 mg

Tar: discarded -

liq (ML): contains Mono Derivatized product: try Prep R-P HPLC, because Analytical HPLC Not had. NM

16/10/07

Right cmp was isolate By RP- prep HPLC (mono derivatized). kept at -20°C For The right. NM.

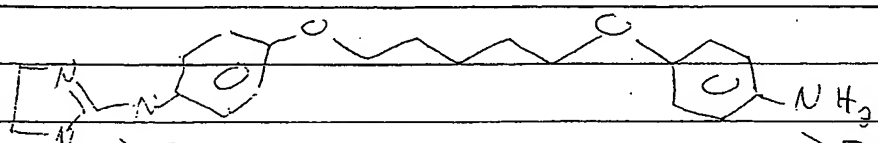
17/10/07 NM

H.943 Transfered into a 25 ml RBF

18/10/07

25.1 mg: ^1H NMR: ^{13}C NMR + MS: 10% after Prep R-P HPLC.

mw: 589.50, 0,043 mmol. 8.5%



TFA TFA

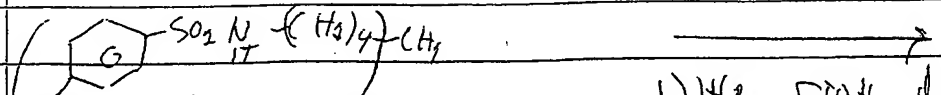
Submitted.

Signature	Read and Understood (print)
	Isabelle Valade
Date (D/M/Y) 18/10/07	Signature 18/10/2007 Date (D/M/Y)

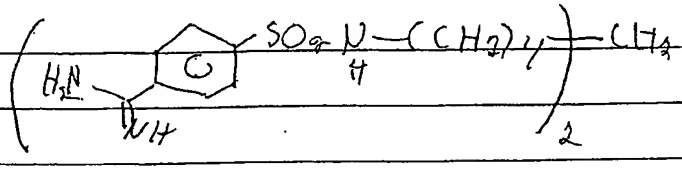
Neurochem Inc.

DM-191-173 15/Oct/2007

Cq, sulfonamide, benzamidine.



① 488.67



521.68]
318

A solution of the Cq - bis sulfonamide ① (318 mg, 0,651 mmol) in EtOH (10 mL) 1,4-dioxane (4 mL) was saturated with dry HCl at 0°C. Stirred at r.t. DM 6/10/07

- solvents (FT IR: no S.M.)
+ 10 mL EtOH, 1,05 g NH_4OAc.
Stirred at r.t (milky). (10:11:00). DM 17/10/07

All: diluted with MeOH: + 1.5 mL HCl conc, : NH_4Cl ↓. Filtered (NH_4Cl) - solvent. Dried in vacuo: product showed MS. ok 21/10/07

0.719. LC more peaks Therm DM-DM-172 DM 24/10/07

Purified by Prep R-P HPLC.
Freeze dried.

Signature	Date (D/M/Y)
C. Mignani	24/10/07

Read and Understood (print)	Signature	Date (D/M/Y)
ISABELLE VALADE	Isabelle Valade	08/11/2007

ydo
3(6)

Neurochem Inc.

DM-191-173 (From p 255) 25/10/02

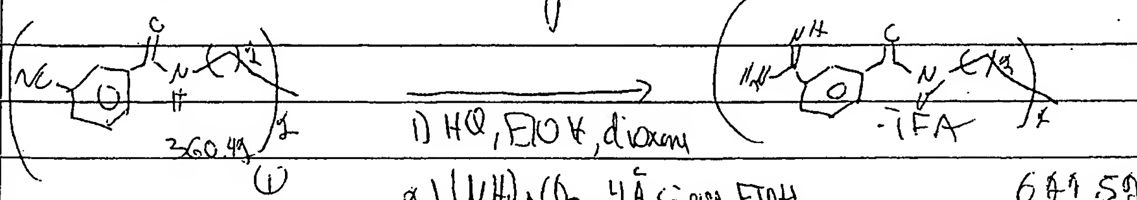
205.2 mg. white solid;
^1H, ^{13}C NMR + MS: Clean.

m.w. 750.73
0.273 m mol
46%.

Submitted.

Signature O. Mignault	Read and Understood (print) ISABELLE VALADE
Date (D/M/Y) 25/10/02	Signature Isabelle Valade Date (D/M/Y) 08/11/2002

C5 - meta bis amidino benzamide



Amidate 525.18

A mixture of (1) (DM-191-901, 480 mg, 1.33 mmol) in 1:1 ethanol/dioxane (20 ml) was cooled with an ice-water bath, saturated with dry HCl. Stirred O/N at r.t. (To: 10:45): start to 5pm. DM.

946.25

04/Dec/02

IR: no C≡N left: concentrated to 1/5 + ether: white solid was collected by filtration, dried in vacuo. 753.9 mg. 1H NMR: good. + 4Å sieve (activated powder) 175 mg) + 20 ml absolute ethanol: 5 min, then + $(NH_4)_2CO_3$ (2.55g). Stirred vigorously at r.t. under N_2. (10:25) DM.

TFA. 679.59
≈ 830 mg

05/Dec/02

Aliquot: MS showed product. Mixture was filtered: solid were rinsed 2 x MeOH (5 ml) + TFA (1.5 ml) - solvent, + MeOH (10 ml) - solvent. White solid was dried in vacuo. 1.99 mostly NH_3.TFA: dissolved in water/MeOH: Purified by RP-Prep HPLC.

goto 022

Signature: D. Migneault
Date (D/M/Y): 05/Dec/02

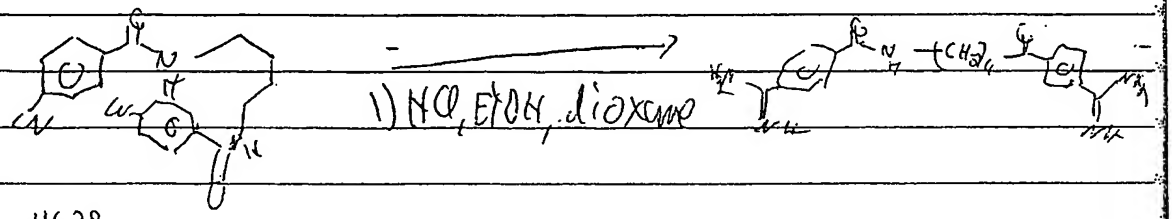
Read and Understood (print): ISABELLE VALADE
Signature: Isabelle Valade
06/12/2002 Date (D/M/Y)

Neurochem Inc.

DM-327-005 015

04/Dec/02

(4 - bis amidino benzamide



346.39

346

453.37

A suspension of (1) (DM-741-303, 460 mg 1.33 mmol) in 1:1 absolute ethanol / 1,4-dioxane (70 mL) at 0°C was saturated with dry HCl. Stirred O/Nat r.t (TD: 10 h35). DM.

05/Dec/02

Aliquot was dried and verified by NMR. FT-IR did not Functionned. +5mL CH_2Cl_2. 70% conversion by ^1H NMR (1 each/mL)

DM

06/Dec/02

Resaturated in HCl. Stirred at r.t. DM.

10/Dec/02

Mixture was now homogenous. NMR: 80% conversion. Cooled with an ice/water bath. Resaturated with dry HCl. Stirred at r.t. DM.

11/Dec/02

No change. — solvent. Residue dried in vacuo.

Signature	Read and Understood (print)
O. Mignani 11/Dec/02	ISABELLE VALADE
Date (D/M/Y)	Signature Isabelle Valade 20/12/2002 Date (D/M/Y)

GoTo 002

022

Neurochem Inc.

DM-937-003 (from p173). 10/Dec/

The crude HPLC purified product (partially dried) was stored at -70°C.

There was too much sln to inject in 1 time. The remaining weight was 0.25g (from 1.9g). Attempt to recrystallize.

43.5838g The purified solid was transferred into a smaller flask, +solvent. White solid was dried in vacuo.

11/Dec/08

1802 mg of a white solid. LH, 13C NMR: pure. To b Submitted, pH 4.5, sol long/ult gentle heat

0.789mmol

Signature	Read and Understood (print)
O. Mignonat	ISABELLE VALADE
11/Dec/08	Isabelle Valade
Date (D/M/Y)	Signature 20/10/2008 Date (D/M

Neurochem Inc.

DM. 227-005 (From 015) 11/Dec/07

-) Problems observed yesterday were the amide. Rx was done.

+ EtOH (30 mL), 4Å sieve (700 mg), $(NH_4)_2CO_3$ (~~9.8g~~) (9.5g). Stirred at r.t (14:01).

DM.
13/Dec/07

qh30'. Mixture was filtered: solids were rinsed with MeOH (10 mL), -solvent.

DM
18/Dec/07

Kept in dessicator until further notice.

Signature	O. Mignonat	Read and Understood (print)	ISABELLE VALADE
Date (D/M/Y)	18/Dec/07	Signature	Isabelle Valade
		Date (D/M/Y)	20/12/2007

go to OD